REMARKS

Claim Rejections Under 35 U.S.C. §101 and § 112, first paragraph

Claims 44-46 and 49-52 stand rejected under 35 U.S.C. §101 as allegedly lacking a specific, substantial and credible asserted utility or a well established utility. Claims 44-46 and 49-52 also stand rejected under 35 U.S.C. 112, first paragraph as allegedly not being supported by either a credible, specific and substantial asserted utility or a well established utility.

The gene amplification assay discloses that genomic DNA encoding PRO269 had a ΔCt value of at least 1-2 units or 2.056 to 3.482 fold amplification in \ eight out of seventeen primary lung tumor samples.

The Patent Office states that the art shows that amplification data for genomic DNA have no bearing on the utility of the encoded polypeptides. In order for PRO269 polypeptides to be overexpressed in lung tumors amplified genomic DNA would have to correlate with amplified mRNA which in turn would have to correlate with amplified polypeptide levels. Allegedly the art discloses that such correlations cannot be presumed.

Applicants disagree with the Examiner's arguments on a number of grounds. The Examiner's arguments will be addressed in order.

(1) The Office Action states that the specification asserts that PRO269 polypeptides are elevated in tumor tissues based on gene amplification results. However, the literature allegedly evidences that this correlation cannot be presumed

In support of the assertion that there is a poor correlation between mRNA levels and protein levels, the Examiner cites Pennica *et al.*, Konopka *et al.* Chen et al., LaBaer, Hu et al., Haynes et al., Gygi et al., Lian et al., Fessler et al. and Greenbaum et al.

Applicants disagree for the reasons previously set forth in their previous responses . The test is whether it is more likely than not that gene amplification results in overexpression of the protein. In order to meet that standard, the Examiner must provide evidence that it is more likely than not that gene amplification does <u>not</u> result in overexpression. Accordingly, Applicants maintain that the Examiner has not met the burden.

On the other hand, Applicants have submitted ample evidence to show that, in general, if a gene is amplified in cancer, it is more likely than not that the encoded protein will be expressed at an elevated level. The articles by Ornitoft *et al.*, Hyman *et al.*, and Pollack *et al.*, collectively teach that in general, gene amplification increases mRNA expression. Accordingly, this rejection is improper.

The Examiner cites Pennica *et al.*, Konopka *et al.* in support of the Office position. References Pennica and Konopka were discussed previously in the Response to Final and Reply Brief and Applicants maintain, for the reasons set forth therein, that they cannot be used to establish a poor correlation between mRNA and protein because these references did not show that, in general, it is more likely than not for mRNA and protein levels not to have a correlation. The reasons were clearly discussed in the Reply Brief. Accordingly, the Examiner has not met the burden of proof.

The Examiner cited Pennica *et al.* as providing general evidence showing lack of correlation between gene (DNA) amplification and elevated mRNA levels. (Office Action page 5). The standard, however, is not absolute certainty. In fact, as noted even in Pennica *et al.*, "[a]n analysis of *WISP*-1 gene amplification and expression in human colon tumors *showed a correlation between DNA amplification and over-expression* . . . ." (Pennica *et al.*, page 14722, left column, first full paragraph, emphasis added). Accordingly, Applicants respectfully submit that Pennica *et al.* teaches nothing conclusive regarding the absence of correlation between amplification of a gene and over-expression of the encoded WISP polypeptide. More importantly, the teaching of Pennica *et al.* is specific to *WISP* genes in human colon tumors. Pennica *et al* has no teaching about lung tumors. Pennica *et al.* has no teaching whatsoever about the correlation of gene amplification and protein expression in general.

The Examiner cited Konopka *et al.* to establish that protein expression is generally not related to gene amplification. Appellants submit that the PTO has generalized a very specific result disclosed by Konopka *et al.* to cover all genes. Konopka *et al.* actually state that "[p]rotein expression is not related to amplification of the ***abl*** gene but to variation in the level of *bcr-abl* mRNA produced from a single Ph^1 template." (See Konopka *et al.*, Abstract, emphasis added). The paper does not teach anything whatsoever about the correlation of protein expression and gene amplification in general, and provides no basis for the generalization that apparently

underlies the present rejection. It is not enough to show that for a particular gene a correlation does not exist. The law requires that the Examiner show evidence that it is more likely than not that such correlation, in general, does not exist. Such a showing has not been made.

The Examiner cites Hu et al., Chen et al., and LeBaer as allegedly supporting the position that there is no correlation between mRNA expression and polypeptide levels.

The Examiner cites Chen et al. as allegedly comparing mRNA and protein expression for a cohort of genes in the same lung carcinomas. "Only 17% of 165 protein spots or 21% of the genes had a significant correlation between protein and mRNA expression levels". Chen et al., allegedly clearly state that "the use of mRNA expression patterns by themselves, however, is insufficient for understanding the expression of protein products' (p. 304) and "it is not possible to predict overall protein expression levels based on average mRNA abundance in lung cancer samples" (pp.311-312).

The manner in which the Chen data was averaged and analyzed is a vastly different manner from that of the instant specification. For example, Chen *et al.* studied expression levels across a set of samples which included a large number of tumor samples (76) and a much smaller group of normal samples (9). The authors determined the global relationship between mRNA and corresponding protein expression using the average expression values for all 85 lung tissue samples. A review of the correlation coefficient data presented in the Chen *et al.* paper indicates that, in fact, Chen teaches that 'it is more likely than not' that increased mRNA expression correlates well with increased protein expression. For instance, a review of Table 1, which lists 66 genes [the paper incorrectly states there are 69 genes listed] for which only one protein isoform is expressed, shows that 40 genes out of 66 had a positive correlation between mRNA expression and protein expression. This clearly meets the test of "more likely than not". Similarly, in Table II , 30 genes with multiple isoforms [again the paper incorrectly states there are 29] were presented. In this case, for 22 genes out of 30, at least one isoform showed a positive correlation between mRNA expression and protein expression. Furthermore, 12 genes out of 29 showed a strong positive correlation [as determined by the authors] for at least one isoform. **No genes showed a significant negative correlation**. It is not surprising that not all isoforms are positively correlated with mRNA expression. Thus, Table II also provides that it is more likely than not that protein levels will correlate with mRNA expression levels.

The same authors in Chen *et al.*, published a later paper, Beer *et al.*, (previously provided) which described gene expression of genes in adenocarcinomas and compared that to protein expression. In this paper they report that "these results suggest that the oligonucleotide microarrays provided reliable measures of gene expression" (page 817). The authors also state, "these studies indicate that many of the genes identified using gene expression profiles are likely relevant to lung adenocarcinoma".

The Examiner offers Hu *et al.* as allegedly analyzing 2286 genes that showed a greater than 1-fold difference in mean expression level between breast cancer samples and normal samples in a microarray. The Examiner states that based on Hu *et al.* the skilled artisan allegedly would not reasonably expect PRO269 protein can be used as a cancer diagnostic.

In their Reply Brief, Applicants discussed the reasons why Hu *et al.* did not establish a *prima facie* case for lack of utility. Applicants rely on the arguments presented therein. The Hu *et al.* reference drew conclusions based upon statistical analysis of information obtained from published literature, and not from experimental data. The statistical analysis by Hu *et al.,* is not a reliable standard because the frequency of citation only reflects the current research interest of a molecule but not the true biological function of the molecule. Accordingly, Hu *et al.* is not sufficient evidence to show that it is likely that PRO269 protein is not overexpressed. The Examiner does not present any meaningful arguments why these criticisms wrong. Accordingly, Hu *et al.* is irrelevant to the instant discussion.

The Examiner states that Dr. LaBaer allegedly made an even stronger statement that reports of mRNA or protein changes of as little as two-fold are not uncommon, and although changes of this magnitude may turn out to be important, most are attributable to disease-independent differences between the samples. Similarly, the comments by LaBaer *et al.* are also based on statistical analysis like Hu, and offers an automated literature mining tool termed MedGene to comprehensively summarize gene-disease relationships. As was argued in the Hu reference, "some molecules may have been underrepresented merely because they were less frequently cited or studied in literature compared to other more well-cited or studied genes." Statistical analysis using literature mining is a very useful tool to assist the researcher in their analysis but may greatly over represent or under represent certain genes and thus their conclusions may not be generally applicable. Accordingly, the statements by LaBaer are

misplaced here.

The Examiner has cited Haynes *et al.*, Gygi *et al.*, Lian *et al.*, Fessler *et al.*, and Greenbaum *et al.*, as allegedly showing that increased mRNA levels do not correlate with increased protein levels in normal tissues.

The Examiner has cited Haynes *et al.* as providing evidence that there is "**no strong correlation** between polypeptide and transcript level. Applicants submit that it is not a legal requirement to establish a necessary or "strong" correlation between an increase in the copy number of the mRNA and protein expression levels that would correlate to the disease state, nor is it imperative to find evidence that DNA amplifications are always associated with overexpression of the gene product. As discussed above, the evidentiary standard to be used throughout *ex parte* examination of a patent application is a preponderance of the totality of the evidence under consideration. Accordingly, the question is whether it is more likely than not that a person of ordinary skill in the art would recognize a positive correlation. Contrary to the Examiner's reading, Haynes *et al.* teaches that "there was a ***general trend*** but no strong correlation between protein [expression] and transcript levels" (Emphasis added).

The Examiner Gygi *et al.* as allegedly concluding "the correlation between mRNA and protein levels was insufficient to predict protein expression levels from quantitative mRNA data. Indeed, for some genes, while the mRNA levels were of the same value, the protein levels varied by more than 20-fold.... Our results clearly delineate the technical boundaries of current approaches for quantitative analysis of protein expression and reveal that simple deduction from mRNA transcript analysis is insufficient."

Applicants submit that Gygi *et al* did not indicate that a correlation between mRNA and protein levels does not exist. Gygi *et al.* only state that the correlation may not be sufficient in **accurately** predicting protein level from the level of the corresponding mRNA transcript (Emphasis added) (see page 1270, Abstract). *Accurate prediction* is not a criteria that is necessary for meeting the utility standards. In fact, contrary to the Examiner's statement, the Gygi data also indicates **a general trend** of correlation between protein [expression] and transcript levels (Emphasis added). For example, as shown in Figure 5, the mRNA abundance of **250-300** copies /cell correlates with the protein abundance of **500-1000** x 10^3 copies/cell. The mRNA abundance of **100-200** copies/cell correlates with the protein abundance of **250-500** x 10^3

copies/cell (emphasis added). Therefore, high levels of mRNA **generally** correlate with higher levels of proteins. In fact, most data points in Figure 5 did not deviate or scatter away from the general trend of correlation. Furthermore, Gygi *et al.* studied yeast cells and not the difference in expression between normal human and lung tumor cells. *Thus*, the Gygi data, meets the "more likely than not standard" and shows that a positive correlation exists between mRNA and protein. Therefore, Applicants submit that the Examiner's rejection is based on a misrepresentation of the scientific data presented in Gygi *et al.*

Applicants refer to the Futcher reference in response to the citation of Gygi. Futcher *et al.* (Mol. Cell. Biol. 19:7357-7368 (1999, previously submitted) analyzed the yeast proteome using 2D gel electrophoresis, gathering quantitative data on protein abundance for about 1,400 spots. This data was compared to mRNA abundance for each gene as determined both by SAGE (serial analysis of gene expression) and by hybridization of cRNA to oligonucleotide arrays. The authors concluded that "**several statistical methods show a strong and significant correlation between mRNA abundance and protein abundance**" (page 7360, col. 2; emphasis added).

The Examiner seems to invalidate the conclusion of Futcher by pointing out that Gygi reaches a different conclusion. Applicants submit that Gygi teaches that a general correlation between mRNA and protein exists.

Moreover, Futcher *et al.* point out that the "different conclusions" of Gygi *et al.* are also partly due to different methods of statistical analysis, and to real differences in data. Futcher *et al.* note that Gygi *et al.* used the Pearson product-moment correlation coefficient (r_p) and point out that "a calculation of r_p is inappropriate" because the mRNA and protein abundances are not normally distributed (page 7367, col. 1). In contrast, Futcher *et al.* used two different statistical approaches to determining the correlation between mRNA and protein abundances. First, they used the Spearman rank correlation coefficient (r_s), an nonparametric statistic that does not require the data to be normally distributed. Using the r_s, the authors found that mRNA abundance was well correlated with protein abundance ($r_s = 0.74$). Applying this statistical approach to the data of Gygi *et al.* **also** resulted in a good correlation ($r_s = 0.59$), although the correlation was not quite as strong as for the Futcher *et al.* data. In a second approach, Futcher *et al.* transformed the mRNA and protein data to forms where they were normally distributed, in

order to allow calculation of an r_p. Two types of transformation (Box-Cox and logarithmic) were used, and **both** resulted in good correlations between mRNA and protein abundance for Futcher *et al.*'s data.

Futcher *et al.* also note that the two studies used different methods of measuring protein abundance. Gygi *et al.* cut spots out of each gel and measured the radiation in each spot by scintillation counting, whereas Futcher *et al.* used phosphorimaging of intact gels coupled to image analysis. Futcher *et al.* point out that Gygi *et al.* may have systematically overestimated the amount of the lowest-abundance proteins, because of the difficulty in accurately cutting out very small spots from the gel, and because of difficulties in background subtraction for small, weak spots.

In addition, Futcher *et al.* note that they used both SAGE data and RNA hybridization data to determine mRNA abundances, which is most helpful to accurately measure the least abundant mRNAs. As a result, while the Futcher data set "maintains a good correlation between mRNA and protein abundance even at low protein abundance" (page 7367, col. 2), the Gygi data shows a strong correlation for the most abundant proteins, but a poor correlation for the least abundant proteins in their data set. Futcher *et al.* conclude that "**the poor correlation of protein to mRNA for the nonabundant proteins of Gygi *et al.* may reflect difficulty in accurately measuring these nonabundant proteins and mRNAs, rather than indicating a truly poor correlation *in vivo***" (page 7367, col. 2; emphasis added). Thus while these lowest abundant proteins do show a poor correlation, this is almost certainly due to the less accurate methods used to measure the abundance of these proteins, and **not** to any actual lack of correlation.

In addition, as Futcher is published later than Gygi, Futcher's conclusion should be considered as the updated view in the art.

The Examiner cites Lian *et al.* as allegedly showing a similar lack of correlation in mammalian (mouse) cells. "The results suggest a poor correlation between mRNA expression and protein abundance, indicating that it may be difficult to extrapolate directly from individual mRNA changes to corresponding ones in protein levels".

Regarding Lian *et al.*, Applicants submit that they only teach that protein expression may not correlate with mRNA level in differentiating myeloid cells and does not teach anything regarding such a lack of correlation for genes in general. In addition, the authors themselves

admit that there are a number of problems with the data presented in this reference. At page 520 of this article, the authors explicitly express their concerns by stating that "[t]hese data must be considered with several caveats: membrane and other hydrophobic proteins and very basic proteins are not well displayed by the standard 2DE approach, and proteins presented at low level will be missed. In addition, to simplify MS analysis, we used a Coomassie dye stain rather than silver to visualize proteins, and this decreased the sensitivity of detection of minor proteins." (Emphasis added). It is known in the art that Coomassie dye stain is a very insensitive method of measuring protein. This suggests that the authors relied on a very insensitive measurement of the proteins studied. The conclusions based on such measurements can hardly be accurate or generally applicable.

The Examiner also asserts that Fessler *et al.*, who examined lipopolysaccharide-activated neutrophilins, "found a 'poor concordance between mRNA transcript and protein expression changes' in human cells." .

Again, as with Lian *et al.,* Fessler *et al.* only examined the expression level of **a few proteins/RNAs** in response to LPS stimulation. Additionally, the PTO has overlooked a number of limitations of the study by Fessler *et al.* For example, as admitted by Fessler *et al.,* protein identification by two-dimensional PAGE is limited to well-resolved regions of the gel, may perform less well with hydrophobic and high molecular weight proteins, and tends to select for more abundant protein species (page 31301, col. 1). Harvesting of the LPS-incubated PMNs at 4 hours may have prevented detection of earlier, **transient changes and may have thereby introduced artificial transcript-protein discordance**. Furthermore, the post-LPS incubation, pre-two-dimensional PAGE cell washes **would be expected to remove secreted proteins from further analysis**. In addition, because protein binding of Coomassie Blue has a limited dynamic range and is typically not linear throughout the range of detection, image analysis of Coomassie Blue-stained protein spots should only be consider as semi-quantitative (see page 31301, col. 1). Again, in this study, low abundance proteins were underrepresented. Therefore, Fessler's study cannot be applied to the present application.

In summary, both Fessler *et al.* and Lian *et al.* have relied on insensitive and inaccurate methods of measuring protein expression levels. The teachings of these two references cannot be relied upon to establish a *prima facie* showing of lack of utility.

The Examiner has cited Greenbaum *et al.* as establishing that mRNA levels cannot predict protein levels. In response, Applicants note that Greenbaum is also comparing the expression of a number of different mRNAs and their corresponding proteins in yeast cells and not comparing the change of expression of specific mRNAs and their corresponding proteins in cancer cells versus normal cells. Accordingly, this reference is also not relevant to the issue at hand. Nevertheless, Greenbaum states that logically "we would assume that those ORFs that show a large degree of variation in their expression are controlled at the transcriptional level. The variability of the mRNA expression is indicative of the cell controlling the mRNA expression at different points of the cell cycle to achieve the resulting and desired protein. **Thus we would expect and we found a high degree of correlation (r-0.89) between the reference mRNA and protein levels for these particular ORFs: the cell has already put significant energy into dictating the final level of protein through tightly controlling the mRNA expression**. (page 117.5 1st column). Furthermore, Greenbaum states : **"we found that ORFs that have higher than average levels of ribosomal occupancy – that is that a large percentage of their cellular mRNA concentration is associated with ribosomes (being translated) – have well correlated mRNA and protein expression levels. (Figure 2)."** Therefore, contrary to the Examiner's assertion, Greenbaum does find high levels of correlation between mRNA and protein expression in yeast cells.

For the reasons given above, Applicants respectfully submit that the Examiner has not established a *prima facie* showing of lack of utility based on the references cited in the Office Action and therefore, the Patent Office has failed to meet its initial burden of proof.

(2) The Examiner now argues that the specification has not identified anything rare, or anything in common among the lung tumor samples in which the PRO269 gene is amplified.

This is incorrect. The specification indicates that PRO269 is overexpressed in various lung tumors. PRO269 was not overexpressed in the other cancers tested, such as colon cancer. Accordingly, PRO269 has utility as a lung tumor marker. It is not a legal requirement that PRO269 distinguish between different types of lung cancers, ie. squamous cell carcinomas, adenocarcinomas etc. The Examiner is applying an incorrect test.

Applicants maintain that this rejection is improper for these reasons and the reasons previously presented and request withdrawal of this rejection.

(3) The Office action indicates that the Goddard declaration has been considered but is not found to be persuasive. The Patent Office previously questioned whether or not a 1.04 to 1.8 ΔCt unit amplification in multiple lung tumors is significant, since half of the lung tumor samples did not show an amplification of the gene encoding PRO269. For the reasons set forth above, Applicants maintain that Applicants do not need to show amplification of PRO269 in every lung tumor cell in order for the results to be significant.

First Applicants note that the case law has clearly established that in considering affidavit evidence, the Examiner must consider all of the evidence of record anew.[1] "After evidence or argument is submitted by the applicant in response, patentability is determined on the totality of the record, by a preponderance of the evidence with due consideration to persuasiveness of argument"[2] Furthermore, the Federal Court of Appeals held in *In re Alton*, "We are aware of no reason why opinion evidence relating to a fact issue should not be considered by an examiner"[3]. Appellants also respectfully draw the Examiner's attention to the Utility Examination Guidelines[4] which state, "Office personnel must accept an opinion from a qualified expert that is based upon relevant facts whose accuracy is not being questioned; it is improper to disregard the opinion solely because of a disagreement over the significance or meaning of the facts offered."

Secondly Applicants have provided two references in which a 2 fold amplification was held to be significant. Applicants note that Orntoft *et al.*, (made of record) states that chromosomal areas with more than a 2-fold gain in DNA showed a corresponding increase in mRNA transcripts. (abstract) Additional supportive teachings were also provided by Pollack *et al.*, (also of record) who studied a series of primary human breast tumors and showed that "62%

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[1] *In re Rinehart*, 531 F.2d 1084, 189 USPQ 143 (C.C.P.A. 1976) and *In re Piasecki*, 745 F.2d. 1015, 226 USPQ 881 (Fed. Cir. 1985).

[2] *In re Alton*, 37 USPQ2d 1578 (Fed. Cir 1966) at 1584 quoting *In re Oetiker*, 977 F.2d 1443, 1445, 24 USPQ2d 1443, 1444 (Fed. Cir. 1992)).

[3] *In re Alton*, *supra*.

[4] Part IIB, 66 Fed. Reg. 1098 (2001).

of highly amplified genes show moderately or highly elevated expression, and DNA copy number influences gene expression across a wide range of DNA copy number alterations (deletion, low-, mid- and high-level amplification), and that on average, a 2-fold change in DNA copy number is associated with a corresponding 1.5-fold change in mRNA levels." (emphasis added) Applicants note that they have shown a more than 2-fold amplification of PRO269 DNA in Example 92.

Accordingly, Applicants request withdrawal of this rejection.

(4) Applicants have previously submitted a Declaration of Dr. Polakis that increased mRNA expression in cancer as compared to normal tissues is correlated with increased protein expression in the same cancerous tissues as compared to normal tissues.

The Examiner indicates that the first Polakis Declaration (Polakis I) is not found persuasive in regard to utility. The Examiner states that there is strong opposing evidence showing that increased mRNA levels are frequently not predictive of increased polypeptide levels.

Applicants again respectfully submit that the standard of proof is not "necessarily correlative" but more likely than not. As Dr. Polakis states, Genentech scientists have found that in approximately **80%** of their observations, an increase in the level of a particular mRNA correlated with changes in the level of protein expressed from that mRNA. Therefore, while the proper legal standard is to show that the existence of correlation between mRNA and polypeptide levels is more likely than not, the showing of approximately 80% correlation for the molecules tested in the Polakis Declaration greatly exceeds this legal standard.

Applicants had previously presented a second Declaration by Dr. Polakis (Polakis II) that presents evidentiary data in Exhibit B. Exhibit B of the Declaration identifies 28 gene transcripts out of 31 gene transcripts (i.e., greater than 90%) that showed good correlation between tumor mRNA and tumor protein levels. As stated in paragraph 5 of Dr. Polakis' Declaration (Polakis II):

> "[a]s such, in the cases where we have been able to quantitatively measure both (i) mRNA and (ii) protein levels in both (i) tumor tissue and (ii) normal tissue, we have observed that in the vast majority of cases, there is a very strong correlation between

increases in mRNA expression and increases in the level of protein encoded by that mRNA."

Accordingly, Dr. Polakis has provided the facts to enable the Examiner to draw independent conclusions.

The Examiner states that the second Polakis Declaration is insufficient to overcome the rejection because it is not clear how the clones appearing in Exhibit B compare to PRO269 or if the results presented in the table were determined by the same methodology as presented in Example 30 of the specification. Applicants respectfully disagree. The Declaration and evidence is being offered in support of the statement that it remains a generally accepted working assumption that increased mRNA levels are more often than not predictive of elevated levels of the encoded protein. The Declaration has probative value regardless of whether PRO269 is included in the table.

The case law has clearly established that in considering affidavit evidence, the Examiner must consider all of the evidence of record anew.[5] "After evidence or argument is submitted by the applicant in response, patentability is determined on the totality of the record, by a preponderance of the evidence with due consideration to persuasiveness of argument"[6] Furthermore, the Federal Court of Appeals held in *In re Alton*, "We are aware of no reason why opinion evidence relating to a fact issue should not be considered by an examiner"[7]. Applicants also respectfully draw the Examiner's attention to the Utility Examination Guidelines[8] which state, "Office personnel must accept an opinion from a qualified expert that is based upon relevant facts whose accuracy is not being questioned; it is improper to disregard the opinion solely because of a disagreement over the significance or meaning of the facts offered."

Applicants provided a Declaration by Dr. Randy Scott ("the Scott Declaration). Dr. Scott was a co-founder of Incyte Pharmaceuticals, Inc., the world's first genomic information business,

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[5] *In re Rinehart,* 531 F.2d 1084, 189 USPQ 143 (C.C.P.A. 1976) and *In re Piasecki,* 745 F.2d. 1015, 226 USPQ 881 (Fed. Cir. 1985).

[6] *In re Alton,* 37 USPQ2d 1578 (Fed. Cir 1966) at 1584 quoting *In re Oetiker,* 977 F.2d 1443, 1445, 24 USPQ2d 1443, 1444 (Fed. Cir. 1992)).

[7] *In re Alton, supra.*

[8] Part IIB, 66 Fed. Reg. 1098 (2001).

and is currently the Chairman and Chief Executive Officer of Genomic Health, Inc., a life sciences company located in Redwood City, California, which provides individualized information on the likelihood of disease recurrence and response to certain types of therapy using gene expression profiling. Based on his more than 15 years of personal experience with the DNA microarray technique and its various uses in the diagnostic and therapeutic fields, and his familiarity with the relevant art, Dr. Scott unequivocally confirms that, as a general rule, there is a good correlation between mRNA and protein levels in a particular tissue.

The Declaration, which is based on Dr. Scott's unparalleled experience with both the microarray technique and its industrial and clinical applications, supports Applicant's position that overexpression of a mRNA correlates with overexpression of protein. Furthermore, Dr. Scott states that the microarray technology is not only mature, reliable and well-accepted in the art, but also has been extensively used in drug development and in diagnosis of various diseases and produced enormous commercial success. Therefore, if an mRNA has been identified to be overexpressed in a certain disease, such as lung cancer, it is more likely than not that the protein product is also overexpressed in the disease.

The Office Action states that Dr. Scott does not base his opinion on any particular facts other than his own considerable experience in the field.

Applicants submit that neither law nor the Utility Guidelines hold that the supporting facts should be narrowly construed as experimental data. Instead, any facts that support the conclusion of the 1.132 Declaration should be accepted by the PTO. Dr Scott has clearly provided sufficient facts to show the commercial success of the microarray technology in drug discovery and further validate the reliability of the presently used microarray technology.

Applicants further submit that the Scott Declaration explicitly addresses the issue of whether there is an mRNA/protein correlation by stating that:

> One reason for the success and wide-spread use of the DNA microarray technique, which has led to the emergence of a new industry, is that generally there is a good correlation between mRNA levels determined by microarray analysis and expression levels of the translated protein. Although there are some exceptions on an individual gene basis, **it has been a consensus in the scientific community that elevated mRNA levels are good predictors of increased abundance of the corresponding translated proteins in a particular tissue**. Therefore, diagnostic markers and drug candidates can be readily and efficiently screened and identified using this technique, without the need to directly measure individual protein

expression levels. (Paragraph 10; Emphasis added).

The commercial data is provided to show that the microarray technology is mature and well-accepted in the art, which is an important issue at hand.

The Examiner states that the mere volume of contradictory publications on this topic speaks to the unpredictability of the issue.

In this regard, Applicants have provided a number of references that gene amplification results in overexpression of protein. Applicants have also provided an extensive list of references which support its position that it is more likely than that if the mRNA is overexpressed the protein will also be overexpressed.

In response to the submitted textbook excerpts by Alberts and Lewin, the Examiner acknowledges that the teachings of Alberts and Lewin support that the initiation of transcription is the most common point for a cell to regulate gene expression. The Examiner asserts, however, that the initiation of transcription "is not the only means of regulating gene expression" according to the teaching of Alberts. (Page 15 of the instant Office Action).

Applicants respectfully submit that the utility standard is not **absolute certainty**. Rather, to overcome the presumption of truth that an assertion of utility by an applicant enjoys, the PTO must establish that it is **more likely than not** that one of ordinary skill in the art would doubt the truth of the statement of utility. Therefore, Applicants **do not need** to establish that transcription initiation is **the only means** of regulating gene expression in order to meet the utility standard. Instead, as long as it is the most common point of regulation, as admitted by the Examiner, it would be more likely than not that a change in the transcription level of a gene gives rise to a change in translation level of a gene. Applicants note that both Alberts and Lewin make clear that it is far more likely than not that protein levels for any given gene are regulated at the transcriptional level. Alberts, for example, states that of all the possible points for regulating protein expression, "[f]or most genes transcriptional controls are paramount." Cell 4th at 379 (emphasis added). In a similar vein, Lewin states that "having acknowledged that control of gene expression can occur at multiple stages, and that production of RNA cannot inevitably be equated with production of protein, it is clear that the overwhelming majority of regulatory events occur at the initiation of transcription." *Genes VI* at 847-848 (emphasis added). Thus, the utility standard is met.

With respect to Applicants' arguments regarding Meric *et al.*, the Examiner asserts that Meric teaches that "gene expression is quite complicated, and is also regulated at the level of mRNA stability, mRNA translation, and protein stability." (Page 16 of the instant Office Action).

Applicants respectfully submit that Meric simply summarizes the translational regulation of cancer cells. Meric indicates that translation initiation is regulated in response to nutrient availability and mitogenic stimulation and is coupled to cell cycle progression and cell growth. Meric further discusses how alterations in translation control occur in cancer. For example, variant mRNA sequences can alter the translational efficiency of individual mRNA molecules. (see Abstract). Meric further teaches that the changes in translational efficiency of a mRNA transcript depend on the mutation of a specific mRNA sequence. (Page 973, column 2 to page 974, column 1). Meric never suggests that the translation of a cancer gene is suppressed in cancer in general, and that therefore, increased mRNA levels will not, in general, yield increased protein levels. To the contrary, Meric teaches that the translation efficiency of a number of cancer genes is enhanced in cancer cells compared to their normal counterparts. For instance, in patients with multiple myeloma, a C-T mutation in the c-myc IRES was identified and found to cause an enhanced initiation of translation (page 974, column 1). Therefore the level of proteins encoded by these genes increases in cancer cells at an even higher magnitude than the corresponding mRNA level. Thus Meric clearly supports Applicants' assertions that it is more likely than not that, in general, changes in mRNA levels are correlated with changes in protein levels.

The Examiner cites Celis *et al.* to the effect that "the number of mRNA copies does not necessarily reflect the number of functional protein molecules." (Page 16 of the instant Office Action).

Applicants respectfully submit that, in their discussion of DNA microarrays and proteomics applied to the same samples, Celis *et al.* cite Orntoft *et al.*, and note that "**in most cases there was a good correlation between transcript and protein levels**" (page 13, col. 1; emphasis added). Celis *et al.* further explain that those few cases which showed apparent discrepancies may have been due to other causes, such as post-transcriptional processing or degradation of the protein, or the choice of methods used to assess protein expression levels.

Celis *et al.* also note that the observation that there is often more change in mRNAs as compared to the proteins may be due to the fact that current technologies detect mainly high abundance proteins, while most of the changes affecting protein levels may involve low abundance proteins. Thus the correlation between mRNA and protein levels may be even higher than typically observed, given these factors.

Applicants further submit that significant correlations between gene and protein expression are most likely to be observed for genes associated with cancer, since as Celis *et al.* note, "transformation resulted in the abnormal expression of normal genes, rather than in the expression of new ones" (page 11, col. 1). Accordingly, alterations in gene amplification or expression are more likely to be associated with altered protein expression in the case of cancer than in other cases where DNA microarrays are used, because, as explained by Celis *et al.*, the alterations in expression levels of certain normal proteins are part of the process that leads to cancer.

With respect to the over one hundred additional references cited in Applicants' Response, the Examiner asserts that "[w]ith the exception of Futcher et al., all of Applicant's newly cited references are directed to the analysis of single genes, or a small group of genes, and therefore do not demonstrate trends found across proteins in general." (Page 16 of the instant Office Action).

Applicants note that the submitted references, which represent experiments conducted by a large number of different research groups, demonstrate a trend of correlation found across proteins in general, and that this trend is confirmed by an overwhelming number of experiments by different researchers, using diverse experimental designs, testing various types of tissues, under numerous biological conditions. Although only a single gene or a small group of genes was tested by each individual study group, the cumulative evidence generated by over one hundred study groups certainly establishes that it is well-accepted in the art that a general mRNA/protein correlation exists.

In a comprehensive study by Orntoft *et al.* (Mol. Cell. Proteomics. 2002; 1(1):37-45) the authors examined gene amplification, mRNA expression level, and protein expression in pairs of non-invasive and invasive human bladder tumors. *Id.* at Abstract. The authors examined 40 well resolved abundant known proteins, and found that "[i]n general there was a highly significant correlation ($p<0.005$) between mRNA and protein alterations. Only one gene showed

disagreement between transcript alteration and protein alteration." *Id.* at 42, col. 2. The alterations in mRNA and protein included both increases and decreases. *Id.* at 43, Table II. Clearly, a correlation in 39 of 40 genes examined supports Applicants' assertion that changes in mRNA level generally lead to corresponding changes in protein level.

The Examiner further asserts that "Orntoft *et al.* concentrated on regions of chromosomes with strong gains of chromosomal material containing clusters of genes... This analysis was not done for PRO269 in the instant specification, and so it is not clear whether or not PRO269 is in a gene cluster in a region of a chromosome that is highly amplified. Therefore, the relevance, if any of Orntoft *et al.* is not clear." (Page 12 of the instant Office Action).

Applicants fail to see how these considerations are relevant to the analysis. Orntoft *et al.* did not limit their findings to only those regions of amplified gene clusters. Further, as discussed in Applicants' previous Responses, Hyman *et al.* and Pollack *et al.*,did gene-by-gene analysis across all chromosomes.

Nagaraja *et al.*, Waghray *et al.*, and Sagynaliev *et al.*

In support of the assertion that "*increases* in mRNA expression and protein samples are not correlated" (page 17 of the instant Office Action; emphasis in original), the Examiner cites three new references, by Nagaraja *et al.*, Waghray *et al.*, and Sagynaliev *et al.*

The Examiner cites Nagaraja *et al.* as allegedly teaching that in comparisons of expression profiles for normal breast compared to breast cancer, "the proteomic profiles indicated altered abundance of fewer proteins as compared to transcript profiles." (Page 17 of the instant Office Action).

Applicants respectfully submit that the fact that many more transcripts than proteins were found to be differentially expressed does not mean that most mRNA changes did not result in correlating protein changes, but merely reflects the fact that expression levels were only measured at all for many fewer proteins than transcripts. In particular, the total number of proteins whose expression levels could be visualized on silver-stained gels was only about 300 (page 2332, col. 1), as compared to the approximately 14,500 genes on the microarray chips for which mRNA levels were measured (page 2336, col. 1). Since the expression levels of so many fewer proteins than transcripts were measured, it is hardly surprising that a smaller absolute

number of proteins than mRNAs were found to be overexpressed, because the protein products of most of the overexpressed mRNAs would not have been among the small number of proteins identified on the gels.

The Examiner next cites Waghray *et al.*, to the effect that "for most of the proteins identified, there was no appreciable concordant change at the RNA level." (Page 18 of the instant Office Action). Applicants emphasize that Applicants are asserting that a measurable change in mRNA level generally leads to a corresponding change in the level of protein expression, not that changes in protein level can be used to predict changes in mRNA level. Waghray *et al.* did not take genes which showed significant mRNA changes and check the corresponding protein levels. Instead, the authors looked at a small and unrepresentative number of proteins, and checked the corresponding mRNA levels. Waghray *et al.* acknowledge that only "[a] relatively small set of genes could be analyzed at the protein level, largely due to the limited sensitivity of 2-D PAGE" (page 1337, col. 1). In particular, while the authors examined the expression levels of 16,570 genes (page 1329, col. 2), they were able to measure the expression levels of only 1031 proteins (page 1333, col. 2). Waghray *et al.* does not teach that changes in mRNA expression were not correlated with changes in expression of the corresponding protein. All Waghray *et al.* state is that "for most of the proteins identified, there was no appreciable concordant change at the mRNA level" (page 1337, col. 2). This statement is not relevant to Applicants' assertion of utility, since Applicants are not asserting that changes in mRNA levels are the only cause of changes in protein levels. Waghray *et al.* do not contradict Applicants' assertion that changes in mRNA expression, in general, correspond to changes in expression of the corresponding protein.

Lastly, the Examiner cites Sagynaliev *et al.*, as allegedly teaching that "it is also difficult to reproduce transcriptomics results with proteomics tools." In particular, the Examiner notes that according to Sagynaliev *et al.*, of 982 genes found to be differentially expressed in human CRC, only 177 (18%) have been confirmed using proteomics technologies. (Page 19 of the instant Office Action).

The Sagynaliev *et al.* reference, titled "Web-based data warehouse on gene expression in human colorectal cancer" (emphasis added), drew conclusions based upon a literature survey of gene expression data published in human CRC, and not from experimental data. While a literature survey

can be a useful tool to assist researchers, the results may greatly over-represent or under-represent certain genes, and thus the conclusions may not be generally applicable. In particular, Applicants note that, as evidenced by Nagaraja *et al.* and Waghray *et al.*, discussed above, the number of mRNAs examined in transcriptomics studies is typically much larger than the number of proteins examined in corresponding proteomics studies, due to the difficulties in detecting and resolving more than a small minority of all expressed proteins on 2D gels. Thus the fact that only 18% of all genes found to be differentially expressed in human CRC have been confirmed using proteomics technologies does not mean that the corresponding proteins are not also differentially expressed, but is most likely due to the fact that the corresponding proteins were not identified on 2D gels, and thus their expression levels remain unknown.

The authors of Sagynaliev *et al.* acknowledge the many technical problems in finding proteomic data for CRC that can be matched to transcriptomic data to see if the two correlate. The authors state that "results have been obtained using heterogeneous samples in particular cell lines, whole tissue biopsies, and epithelial cells purified from surgical specimens." However, "Results obtained in cell lines do not allow accurate comparison between normal and cancer cells, and the presence/absence of proteins of interest has to be confirmed in biopsies." (Page 3072, left column.) In particular, the authors specifically note that "only a single study [1] provided differential display protein expression data obtained in the human patient, using whole tissue biopsy." (Page 3068, left column, second paragraph; *see also*, Table 2.) The examiner also notes and the authors state, "For CRC, there is no publication comparing mRNA and protein expression for a cohort of genes." (Page 3077, left column, last paragraph, emphasis added.)

Applicants further note that Table 2 shows that 6 out of 8 published proteomics studies were done using 2-D PAGE. However, the authors state that "2-D PAGE or 2-D DIGE have well-known technological limitations ... even under well-defined experimental conditions, 2-D PAGE parallel analysis of paired CRC samples is hampered by a significant variability." (Page 3077, left column, third paragraph.) Therefore, Applicants respectfully submit that it is well known in the art that there are problems associated with selecting only those proteins detectable by 2D gels.

The Patent Office has failed to meet its initial burden of proof that Applicant's claims of utility are not substantial or credible. The arguments presented by the Examiner in combination with the previously cited Hu, Chen, Anderson, Lian, Fessler and Greenbaum papers, as well as

the newly cited Nagaraja, Waghray, and Sagynaliev papers, do not provide sufficient reasons to doubt the statements by Applicants that PRO269 has utility. As previously discussed, the law does not require the existence of a "necessary" correlation between mRNA and protein levels. Nor does the law require that protein levels be "accurately predicted." According to the authors themselves, the data in the above cited references confirm that there is a general trend between protein expression and transcript levels, which meets the "more likely than not standard" and show that a positive correlation exists between mRNA and protein. Therefore, Applicants submit that the Examiner's reasoning is based on a misrepresentation of the scientific data presented in the above cited reference and application of an improper, heightened legal standard. In fact, contrary to what the Examiner contends, the art indicates that, if a gene is overexpressed in cancer, it is more likely than not that the encoded protein will also be expressed at an elevated level.

Lilley *et al.*, Wildsmith *et al.* and King *et al.*

The Examiner next asserts that "the state of the art, as evidenced through textbooks and review papers, clearly establishes that polypeptide levels cannot be accurately predicted from mRNA levels." (Pages 19-20 of the instant Office Action). In support of this assertion, the Examiner cites textbook excerpts by Lilley *et al.* and Wildsmith *et al.*, and an article by King *et al.* In particular, the Examiner cites Lilley *et al.* to the effect that "the extrapolation that changes in transcript level will also result in corresponding changes in protein amount or activity *cannot always be made*." The Examiner cites Wildsmith *et al.* to the effect that "the gene expression data obtained from a microarray *may differ* from protein expression data." Finally, the Examiner cites King *et al.* to the effect that "mRNA expression studies should be accompanied by analysis at the protein level."

Applicants reiterate that the evidentiary standard to be used throughout *ex parte* examination of a patent application is a preponderance of the totality of the evidence under consideration. Accordingly, in order to overcome the presumption of truth that an assertion of utility by the applicant enjoys, the Examiner must establish that **it is more likely than not** that one of ordinary skill in the art would doubt the truth of the statement of utility. **The standard is not absolute certainty.** The law requires only that one skilled in the art should accept that such a correlation is **more likely than not to exist**. The law does not require a "necessary" correlation

between mRNA and protein levels. Nor is it required that protein levels can be "accurately predicted" from mRNA levels. Nowhere in these papers do the authors suggest that it is more likely than not that altered mRNA levels do not correlate with altered protein levels. On the contrary, statements such as "the extrapolation that changes in transcript level will also result in corresponding changes in protein amount or activity cannot *always* be made" imply that the mRNA/protein correlation exits in most cases.

Applicants further note that the cited papers disclose a number of successful examples of microarray applications in human disease study, which further validate Applicants' assertions. For example, Wildsmith *et al.* points out that

> one area of rapid progress using microarray technology is the increased understanding of cancer. Molecular pathologies are subgrouping cancers of tissues such as blood, skin, and breast, based on differential gene expression patterns. For example, within a small group of breast cancer tissue samples, Perou *et al.* distinguished two broad subgroups representing those expressing or alternatively lacking expression of the oestrogen receptor- α- gene. The work was not conclusive, but never has progress in this field been so rapid when compared with the previous methods of gene identification. Another example of the impact of this technology is in the identification of two biomarkers for prostate cancer, namely hepsin and PIM1 (Dhanasekaran *et al.*, 2001). Microarray technology has also accelerated the understanding of the molecular events surrounding pulmonary fibrosis. Specially, two distinct clusters of genes associated with inflammation and fibrosis have been identified in a disease where, for years, the pathogenesis and treatment have remained unknown (Katsuma *et al.*, 2001). (Page 284).

King *et al.* disclose that microarray technology offers tremendous advantages in human disease study. For example, the authors state that "microarrays can be expected to prove extremely valuable as tools for the study of the generic basis of complex diseases. The ability to measure expression profiles across entire genomes provides a level of information not previously attainable…Microarrays make it possible to investigate differential gene expression in normal vs. diseased tissue, in treated vs. non-treated tissue, and in different stages during the natural course of the disease, all on a genomic scale. Gene expression profiles may help to unlock the molecular basis of phenotype, response to treatment, and heterogeneity of disease." (Page 2287, column 3).

Bork *et al.*

The Examiner also refers to a paper by Bork *et al.* (Page 20 of the instant Office Action). Bork et al is currently not of record in the application. If the Examiner intends to rely on Bork

the Examiner should make Bork of record on a PTO-892 form. Bork *et al.* comments generally about high-throughput technologies (which include microarrays) and in fact, validates the positive potential of such technologies by admitting that such technologies "often reveal important general trends that are impossible to realize with classical, low-throughput experimental methods, yet provide fewer insights into specific, molecular detail (see page 1, column 1, line 3-8 of the Bork article). This article comments on the limitations in the "total knowledge base" of protein function. Bork further quotes Anderson *et al.*'s coefficient of 0.48 as the correlation between mRNA and protein expression. Applicants maintain that a 0.48 correlation value (about 50%) supports the contention that it is "more likely than not" that protein expression correlates well with mRNA expression. Therefore, Bork supports the Applicants' position that changes in mRNA levels are generally correlated with changes in protein levels.

Haynes *et al.*

The Examiner cites Haynes *et al.* to the effect that "[p]rotein expression levels are not predictable from the mRNA expression levels." (Page 20 of the instant Office Action). Applicants respectfully point out that Haynes *et al.* never indicate that the correlation between mRNA and protein levels does not exist. Haynes *et al.* only state that "protein levels cannot be *accurately* predicted from the level of the corresponding mRNA transcript" (See page 1863, under Section 2.1, last line, emphasis added). This result is expected, since there are many factors that determine translation efficiency for a given transcript, or the half-life of the encoded protein. Not surprisingly, Haynes *et al.* concluded that protein levels cannot always be accurately predicted from the level of the corresponding mRNA transcript in a single cellular stage or type when looking at the level of transcripts across different genes.

Importantly, Haynes *et al.* did not say that for a single gene, a change in the level of mRNA transcript is not positively correlated with a change in the level of protein expression. Applicants have asserted that increasing the level of mRNA for a particular gene leads to a corresponding increase for the encoded protein. Haynes *et al.* did not study this issue and says absolutely nothing about it. One cannot look at the level of mRNA across several different genes to investigate whether a change in the level of mRNA for a particular gene leads to a change in the level of protein for that gene. Therefore, Haynes *et al.* is not inconsistent with or

contradictory to the utility of the instant claims, and offers no support for the PTO's rejection of Applicants' asserted utility.

Furthermore, Applicants note that contrary to the Examiner's statement, Haynes teaches that "*there was a general trend* but no strong correlation between protein [expression] and transcript levels" (See page 1863, under Section 2.1, emphasis added). For example, in Figure 1, there is a positive correlation between mRNA and protein amongst *most* of the 80 yeast proteins studied but the correlation is not linear, hence the authors suggest that one cannot *accurately* predict protein levels from mRNA levels. In fact, very few data points deviated or scattered away from the expected normal or showed a lack of correlation between mRNA: protein levels. Thus, the Haynes data meets the "more likely than not standard" and shows that a positive correlation exists between mRNA and protein. Therefore, Applicants submit that the Examiner's rejection is based on a misrepresentation of the scientific data presented in Haynes *et al.*

Haynes *et al.* may teach that protein levels cannot be "accurately predicted" from mRNA levels in the sense that the exact numerical amounts of protein present in a tissue cannot be determined based upon mRNA levels. Applicants respectfully submit that the PTO's emphasis on the need to "accurately predict" protein levels based on mRNA levels misses the point. The asserted utility for the claimed polypeptides is in the diagnosis of cancer. What is relevant to use as a cancer diagnostic is relative levels of gene or protein expression, not absolute values, that is, that the gene or protein is differentially expressed in tumors as compared to normal tissues. Applicants need only show that there is a correlation between mRNA and protein levels, such that mRNA overexpression generally predict protein overexpression. A showing that mRNA levels can be used to "accurately predict" the precise levels of protein expression is not required.

Madoz Gurpide *et al.*

The Examiner cites Madoz Gurpide *et al.* to the effect that "[f]or most of the published studies, it is unclear how well RNA levels reported correlate with protein levels." (Page 20 of the instant Office Action). Applicants respectfully point out that Madoz Gurpide *et al.* state only that is it "unclear" how well RNA levels reported correlate with protein levels, not that the levels do not correlate. Madoz Gurpide *et al.* also acknowledge that DNA microarray studies "**justify the use of this technology for uncovering patterns of gene expression that are clinically informative**" (page 53; emphasis added).

Applicants respectfully submit that while proteomics is indeed a complementary technology to DNA microarrays, this does not mean that proteomic experiments are required in addition to measurements of mRNA levels to determine protein expression. The cited papers make clear that proteomic techniques are useful to obtain information beyond expression levels, such as the protein's activation state, posttranslational modifications, and subcellular localization. Haynes *et al.*, as quoted in the instant Office Action, states that "only the direct analysis of mature protein products can reveal their correct identities, their relevant state of modification and/or association, and their amounts." (Page 20 of the instant Office Action).

While this additional information may be useful in elucidating the detailed biological function of a protein, it is not required to establish utility of a protein as a marker for cancer. The PRO269 polypeptide and the claimed antibodies that bind it can be used in cancer diagnosis without any knowledge regarding the function or cellular role of the polypeptide. Applicants submit that the law clearly states that "it is not a requirement of patentability that an inventor correctly set forth, or even know, how or why the invention works." *Newman v. Quigg*, 11 U.S.P.Q.2d 1340 (Fed. Cir. 1989). Accordingly, the disclosure or identification of the mechanism by which PRO269 is associated with cancer is not required in order to establish the patentable utility of the claimed PRO269 polypeptides. Thus while Madoz-Gurpide *et al.* note that it is "more difficult to develop an understanding of disease at a mechanistic level using DNA microarrays," (page 53) this is not relevant to Applicants' assertions of utility, since, as discussed above, it is not necessary to understand how or why an invention works in order to demonstrate utility.

In response to Applicants' previously submitted 150 supporting references, the Examiner further asserts that the majority of the newly cited references, such as Wang et al., Maruyama et al., Munaut et al., Rudlowski et al. and Bea et al., are drawn to genes whose encoded protein were previously known or suspected to be overexpressed or under-expressed in cancer, and that are involved with cell proliferation, differentiation and/or cell adhesion/migration, in which expression of protein is important in the development and progression of the cancer. In contrast to these studies, PRO269 is not a putative oncogene and the function of the encoded protein is not known. (Pages 21-24 of the instant Office Action).

Applicants submit that, just as in Wang *et al.,* Maruyama *et al.,* Manuat *et al.,* Rudlowski

et al. and Bea *et al.*, PRO269 is shown in the amplification assay to be "involved in cancer." For claiming utility for a novel molecule, there is no requirement that the specification provide a physiological or biochemical explanation regarding how the claimed polypeptides provide the useful function of being diagnostic of colon cancer. As discussed above, "it is not a requirement of patentability that an inventor correctly set forth, or even know, how or why the invention works."[9] In addition, Applicants note that the important question is whether or not the claimed invention is useful, and not whether or not Applicants have provided the best method for its operation as a cancer diagnostic. For instance, as stated by the Federal Circuit: "[a]n invention need not be the best or only way to accomplish a certain result, and it need only be useful to some extent and in certain applications: '[T]he fact that an invention has only limited utility and is not operable in certain applications is not grounds for finding lack of utility.' *Envirotech Corp. v. Al George, Inc.* 730 F.2d 753,762, 221 U.S.P.Q. 473,480 (Fed. Cir. 1984).

Applicants emphasize that Applicants rely on the diagnostic utility for the presently claimed PRO269 polypeptide. While the structural/function/homology may be useful in elucidating the detailed biological function of a protein and seeking therapeutic agents for human diseases, such as cancer, it is not required to establish utility of a protein as a diagnostic marker for cancer. The claimed PRO269 polypeptides can be used in cancer diagnosis without any knowledge regarding the function or cellular role of the polypeptides. Accordingly, the disclosure or identification of the mechanism by which PRO269 is associated with cancer is not required in order to establish the patentable utility of the claimed PRO269 polypeptides.

Finally, Applicants note that in each of the references cited, the expression level of the protein correlated with the expression level of the mRNA and the change in expression level was correlated with cancer. This supports Applicants position.

Godbout et al.

With respect to the over one hundred additional references cited by Applicants', the Examiner asserts that only a single reference, that by Godbout *et al.*, is relevant to the issue of whether gene amplification is correlated with increased mRNA and protein expression levels.

[9] *In re Cortwright*, 165 F.2d 1353, 1359, 49 U.S.P.Q.2d 1464, 1469 (Fed. Cir. 1999), citing *Newman v. Quigg*, 877 F.2d 1575, 1581, 11 U.S.P.Q.2d 1340, 1345 (Fed. Cir. 1989).

(Page 2 of the October, 13, 2006 Office Action). Applicants have acknowledged that the new references focus on the correlation between mRNA expression and protein expression levels, and for the most part do not examine gene amplification. However, those few references that actually looked at gene amplification **did** find a correlation between gene amplification and increased mRNA and protein expression levels. Applicants further respectfully submit that, Bea *et al.* investigated gene amplification, mRNA expression, and protein expression of the putative oncogene BMI-1 in human lymphoma samples, and supports Applicants' assertion that gene amplification is correlated with both increased mRNA and protein expression.

The Examiner further asserts that Godbout *et al.* teaches that "it is generally accepted that co-amplified genes are not over-expressed unless they provide a selective growth advantage to the cell.". Applicants respectfully submit that the passage cited by the Examiner is based upon two references from 1987 and 1992. In contrast, Applicants have made of record three more recent references, published in 2002, by Orntoft *et al.*, Hyman *et al.*, and Pollack *et al*, which collectively teach that in general, gene amplification increases mRNA expression. Applicants submit that these more recent references must be acknowledged as more accurately reflecting the state of the art regarding the correlation between gene amplification and transcript expression than the references cited by Godbout *et al.*

The Examiner also cites an additional paper by Li *et al.* as teaching that "68.8% of the genes showing over-representation in the genome did not show elevated transcript levels.". Applicants respectfully point out that Li *et al.* acknowledge that their results differed from those obtained by Hyman *et al.* and Pollack *et al.* (of record), who found a substantially higher level of correlation between gene amplification and increased gene expression. The authors note that "[t]his discordance may reflect methodologic differences between studies or biological differences between breast cancer and lung adenocarcinoma" (page 2629, col. 1). In fact, as explained in the Supplemental Information accompanying the Li article (copy enclosed as Exhibit A), genes were considered to be amplified if they had a copy number ratio of at least 1.40. As discussed in Applicants' previous responses, and in the Goddard Declaration of record, an appropriate threshold for considering gene amplification to be significant is a copy number of at least 2.0. As discussed above the PRO269 gene showed at least a 2 fold amplification in eight different lung tumors, thus meeting this standard. It is not surprising that, by using a

substantially lower threshold for considering a gene to be amplified, Li *et al.* would have identified a number of genes that were not in fact significantly amplified, and therefore did not show any corresponding increase in mRNA expression. The results of Li *et al.* therefore do not disprove that a gene with a substantially higher level of gene amplification, such as PRO269, would be expected to show a corresponding increase in transcript expression.

Taken together, although there are some examples in the scientific art that do not fit within the central dogma of molecular biology that there is a correlation between polypeptide and mRNA levels, these instances are exceptions rather than the rule. In the majority of amplified genes, the teachings in the art overwhelmingly show that gene amplification influences gene expression at the mRNA and protein levels. Therefore, one of skill in the art would reasonably expect in this instance, based on the amplification data for the PRO269 gene, that the PRO269 polypeptide is concomitantly overexpressed. Thus, Applicants submit that the PRO269 polypeptides and antibodies have utility in the diagnosis of cancer and based on such a utility, one of skill in the art would know exactly how to use the polypeptide for diagnosis of cancer.

Accordingly, this rejection under 35 U.S.C. §101 and §112, first paragraph, should be withdrawn.

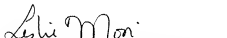
CONCLUSION

For the reasons given above, Applicants submit that the gene amplification assay disclosed in Example 92 of the specification, and the advanced state of the art in oncology, provide at least one patentable utility for the PRO269 polypeptides of Claims 44-46 and 49-52, and that one of ordinary skill in the art would understand how to use the claimed polypeptides and would have found such testing routine and not 'undue.' Therefore, Claims 44-46 and 49-52 meet the requirements of 35 U.S.C. §101 and 35 U.S.C. §112, first paragraph.

Please charge any additional fees, including any fees for additional extension of time, or credit overpayment to Deposit Account No. **08-1641** (referencing Attorney's Docket No. **39780-1618 P2C33**).

Respectfully submitted,

Date: May 1, 2007

By: 
Leslie A. Mooi (Reg. No. 37,047)

HELLER EHRMAN LLP
275 Middlefield Road
Menlo Park, California 94025
Telephone: (650) 324-7000
Facsimile: (650) 324-0638

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